Crystal Structure of Smad3 MH2 Domain

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Abstract No. Qin2834
Beamline(s) **X25**

Introduction: Smad3 functions as a signal transducer and transcriptional regulator of the TGF-beta signaling pathway. Smad3 is activated upon receptor kinase-mediated phosphorylation at the C-terminal tail. Phosphorylated Smad3 forms a complex with Smad4, and the heteromeric complex enters the nucleus to regulate transcription of target genes. To understand the structural basis of Smad3 function, we crystallized the conserved C-terminal MH2 domain of Smad3, which plays important role in several signaling steps.

Methods and Materials: The Smad3 MH2 domain was cloned by PCR and expressed as a GST fusion protein in *E. coli*. The protein was purified by standard chromatography procedures using glutathione sepharose and DEAE sepharose. The protein was crystallized by the hanging drop vapor diffusion technique using well solution containing 20% PEG, 100 mM Tris pH7.4 and 100 mM NaCl. The crystals were flash frozen in liquid nitrogen using the cryo tong. The data were collected at beam line X23B at a detector distance of 100 mm. The oscillation angle was 1 degree and the exposure time was 30 seconds. The data were indexed and integrated at the beam line using Denzo/Scalepack. The data were of excellent quality to resolution of 2 Å.

Results and **Conclusions**: The structure is being determined by molecular replacement using the Smad2 MH2 domain as a search model. We expect the structure will provide insight into critical steps of the signaling mechanism such as recruitment of Smad3 to the receptor kinase for phosphorylation, phosphorylation-induced activation and basis of transcrptional regulation.

Acknowledgments: We would like to thank Dr. Berman for help with the data collection. The work is supported by the Sidney Kimmel foundation.